Fast-Fusion™ Cloning Kit

For rapid and effective cloning of PCR products

Cat. No. FFPC-C020 (20 reactions)
Cat. No. FFPC-C060 (60 reactions)

User Manual

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User Manual

Fast-Fusion™ Cloning Kit

I. Introduction

Fast-Fusion™ Cloning Kit provides a rapid method for cloning your PCR product. In just 15 minutes at room temperature, any PCR fragment can be cloned into your linearized vector at will. After a simple clean up step, a PCR-generated DNA fragment or other purified DNA fragment can be joined to a vector with overlapping ends (Fig.1). Up to eight DNA fragments can be joined together in a single reaction. Well-prepared vectors generate almost 100% positive clones.

There is no restriction site required at the junction site. Therefore, your fragment of interest can be inserted at any position in the vector. The linearized vector can be generated by either PCR or restriction enzyme digestion. The PCR products can be produced by either Taq DNA polymerase or other high fidelity DNA polymerase.

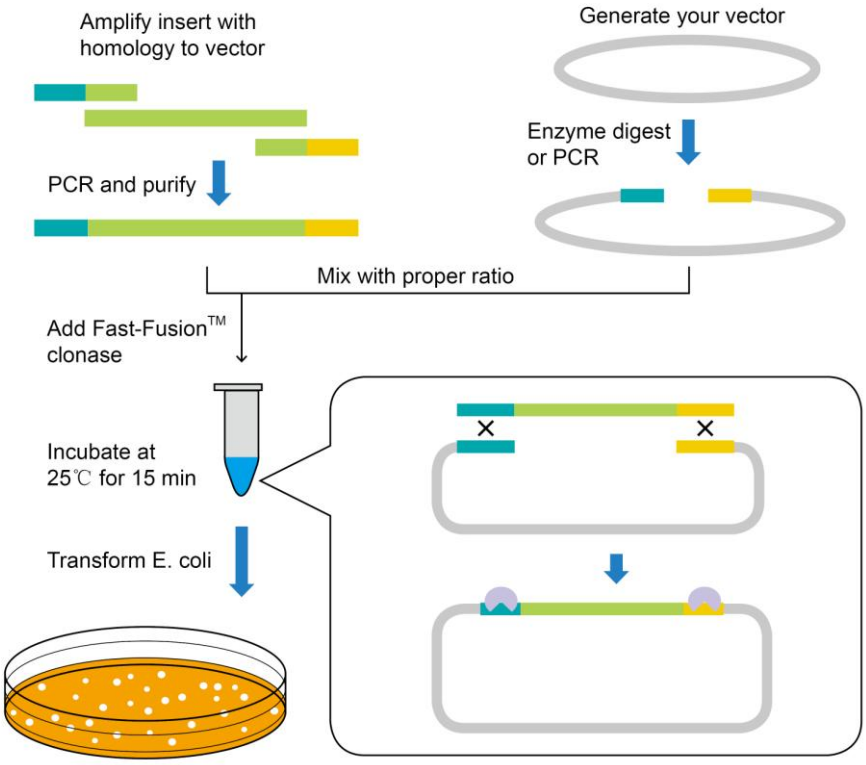


Fig.1. Experimental workflow of single fragment insertion into a vector using the GeneCopoeia Fast-Fusion™ Cloning Kit.

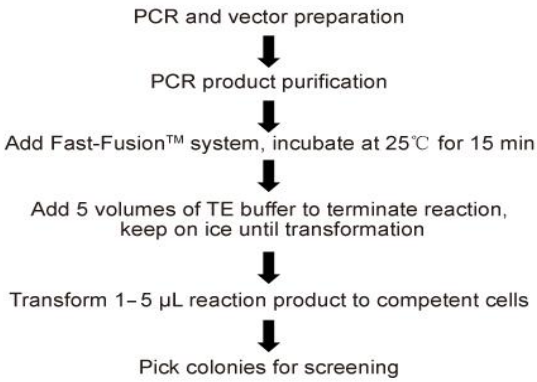
Work principle

The GeneCopoeia Fast-Fusion™ Cloning Kit inserts the fragment into the vector using two simultaneous steps: a. homology recognition; b. strand exchange and redundant strand degradation. The gaps remaining in the recombinant strands will be repaired by *E. coli* after transformation.

Key Advantages

- Fast and simple—1 minute for operation and 15 minutes for incubation at room temperature.
- High efficiency—Greater than 90% of colonies after transformation contain the correct insert(s).
- High adaptability—No restriction site or recombination site needed, insert fragments generated by either PCR or restriction enzyme digestion can be used.
- Flexibility—Multiple inserts can be assembled in one reaction. Suitable for multi-site mutagenesis.
- Seamless construction—Final constructs have no extra base pairs remaining.

Protocol overview



II. Contents and Storage

Contents and storage recommendations for the GeneCopoeia Fast-Fusion™ Cloning Kit (Cat.Nos.FFPC-C020 and FFPC-C060) are provided in the following table.

Contents	Quantity	Shipping temperature	Storage temperature
Fast-Fusion™ Clonase	1 × 20 μL 3 × 20 μL	Dry ice or ice pack	-20°C Stable for at least 12 months
10 × Clonase Buffer	1 × 20 μL 3 × 20 μL	Dry ice or ice pack	-20°C Stable for at least 12 months
QP Reagent	1 × 500 μL 3 × 500 μL	Dry ice or ice pack	-20°C Stable for at least 12 months
TE Buffer	1 × 500 μL 3 × 500 μL	Dry ice or ice pack	-20°C Stable for at least 12 months
Linearized pUC19 (50 ng/μL)	1 × 10 μL 3 × 10 μL	Dry ice or ice pack	-20°C Stable for at least 12 months
Positive Insert (100 ng/μL)	1 × 10 μL 3 × 10 μL	Dry ice or ice pack	-20°C Stable for at least 12 months

Additional materials required but not provided

Clonable plasmid vector
Taq or other high fidelity DNA polymerases
DNA quantitation standard
Restriction enzymes
Gel purification kit
Competent cells for transformation
S.O.C. medium
LB plates with antibiotics

III. Key Steps

1. **Vector preparation:** A well-prepared vector can reduce your screening time. Single enzyme-digested vectors will self-ligate resulting in a high background of plasmids lacking inserts following transformation. The best way to avoid this is to digest with two restriction enzymes, followed by gel purification of the vector backbone. For PCR-generated vectors, we recommend digestion with Dpn I which will destroy plasmids that have been Dam methylated by replication in *E. coli*. Transform 50-100 µL of competent cells with 5-10 ng linearized vector as a negative control to determine the transformation background.

2. **Primer design:** Primer design is critical for successful Fast-Fusion™ cloning. Homology must present at the ends you want to fuse, e.g. vector and insert (or multiple inserts). For homologies less than 15 bp, the transformation efficiency will vary depending on DNA structure (Fig.2). GeneCopoeia strongly recommends including more than 15 bp of homology at each end for best results. Check your homology following the guidelines below (Fig.3)

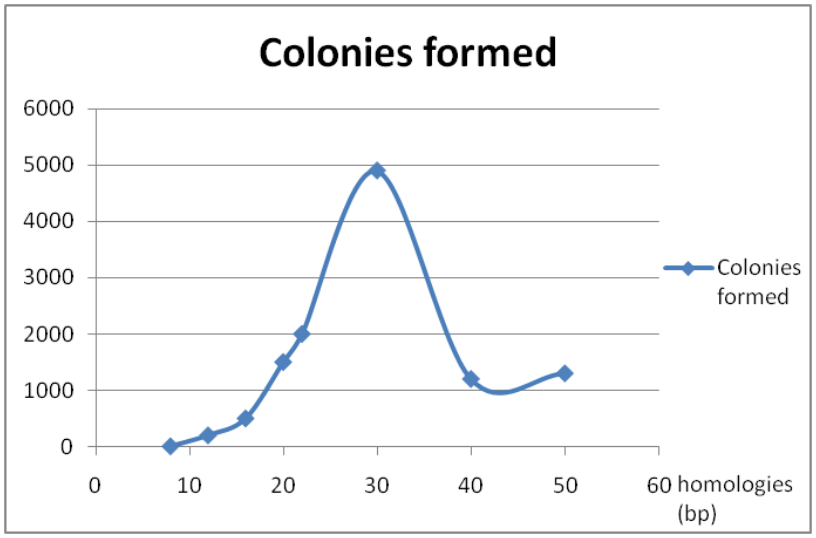


Fig.2. Homologies affect cloning efficiency. The number of colonies formed is calculated from 5 ng of pUC19 vector transformed after standard Fast-Fusion™ reactions with inserts of indicated homologies (Competent cells efficiency: 2×10^9cfu/ug).

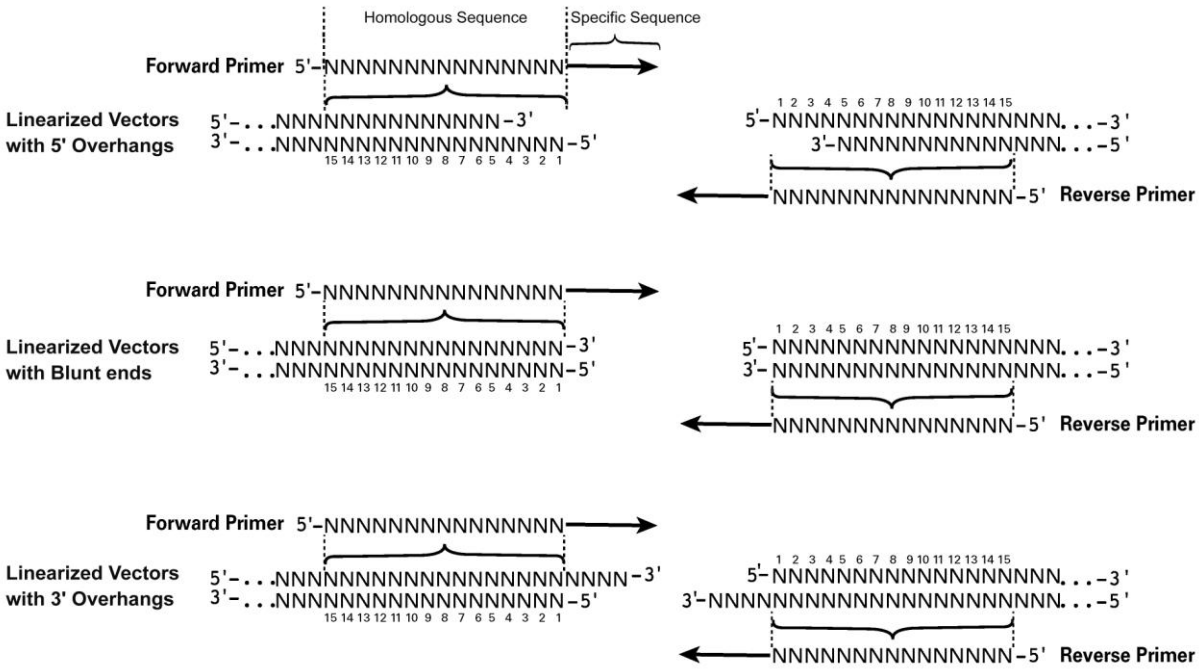


Fig.3. Primer with 15 bp homology in different vector ends.

3. **PCR amplification and purification:** Taq and other high fidelity DNA polymerases are all suitable for generating DNA fragments for Fast-Fusion™ cloning. After PCR, analyze PCR products by electrophoresis on an agarose/EtBr gel. The QP reagent can be used when only a single band is present (Fig.4). Gel purification is strongly recommended when nonspecific amplification is evident. Quantify the purified fragments by measuring against a known DNA standard running in parallel.

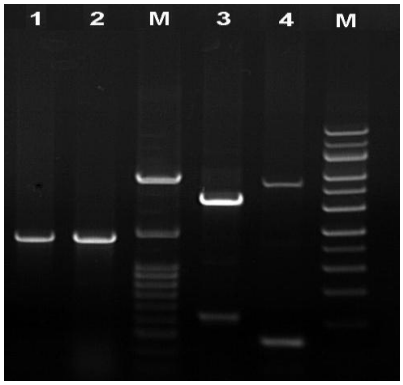


Fig.4. PCR inserts for Fast-Fusion™ cloning.

Lane 1: Insert PCR purified by QP reagent.

Lane 2: Insert PCR without purification.

Lane3, 4: Nonspecific amplification in PCR reaction.

4. **Use of QP reagent:** The QP reagent can precipitate double stranded DNA longer than 100 bp, excluding dNTPs, primers and most of the polymerase.

(1) Invert the QP reagent tube several times before use.

(2) For 50 µL of PCR product, add TE buffer to 100 µL, followed by addition of 50 µL QP reagent. Mix thoroughly by vortexing for 5 seconds.

(3) Centrifuge the mixture at 15,000×g for 15 minutes, and discard the supernatant. Re-centrifuge the tube for 10 seconds and remove all the remaining liquid at the bottom.

Note: To obtain better precipitation efficiency for DNA molecules shorter than 200 bp, incubate at 4°C for at least 30 minutes before centrifugation.

(4) Re-suspend the DNA by adding 10-20 µL diluted TE Buffer (Dilute TE Buffer to 10% with ddH_2O).

IV. Cloning Reaction and Transformation Procedure

1. Cloning Reaction

(1) Set up the following cloning reaction on ice.

Component	Volume / Amount
10 × Clonase Buffer	1 µL
Linearized vector	20-100 ng
Insert	20-200 ng
ddH_2O	Add ddH_2O to **9 µL**

Note: The recommended molar ratio of insert to vector should be 2-5**:**1. Use the table below as a guide.

vector		insert	
Length	**Quantity**	**Length**	**Quantity**
3k bp	20-50 ng	200-2000 bp	100 ng
5k bp	40-80 ng	2k-5k bp	100-150 ng
9k bp	50-100 ng	> 5k bp	150-200

(2) Add **1 µL** Fast-Fusion™ clonase to the reaction and mix by tapping the tube.

(3) Spin down briefly to collect the reagents at the bottom of the tube. Incubate at 25°C for 15 minutes.

(4) Add 40 µL TE Buffer to terminate the reaction, or place the tube on ice until transformation.

2. Transformation

Transform competent *E. coli* cells with your Fast-Fusion™ products using the provided protocol (below) or by following the manufacturer's instructions. GeneCopoeia recommends using high-efficiency competent cells (>10^8 cfu/µg).

(1) Transfer 1 to 5 µL of reaction mixture (5-25 µL after dilution with TE Buffer) to 100 µL chemically competent cells. Incubate on ice for 30 minutes.

Note: 1 µL is usually sufficient for single-insert cloning. Increase volume for multi-insert assembly.

(2) Heat-shock the cells for exactly 30 seconds at 42°C without shaking, then immediately place the tubes on ice for 2 minutes.

(3) Add 400 µL of room temperature S.O.C. medium to the cells.

(4) Cap the tubes and incubate at 37°C for 1 hour with or without shaking.

(5) Spread 50 to 500 µL cells from each tube on pre-warmed LB plates containing the appropriate antibiotics.

(6) Incubate plates at 37°C overnight.

(7) Pick colonies for analysis.

V. Troubleshooting

The tables below address two main problems encountered during Fast-Fusion™ cloning, along with their possible causes and suggested solutions. Please perform the control reactions to confirm that the kit is working properly before you call us for help.

1. Problem: Few or no colonies obtained from transformation.

Possibility	Solution
Competent cells' efficiency is insufficient	Check with control reaction. There should be at least 100 colonies for competent cells over 10^8 cfu/µg
DNA solution impurity	Purify the DNA by gel purification, etc.
Low DNA concentration in reaction	Check with known concentration DNA standards, concentrate the DNA to over 20 ng/µL.
Primer sequences are incorrect	Check your primers to ensure the products provide corresponding bases of homology with their neighbors.
Not enough homology	Homologies over 20 bp give best results. Don't use less than 12 bp if your competent cell efficiency is below 10^9 cfu/µg.
Incomplete 3' ends generated by PCR, especially for proofreading polymerases	Increase the elongation time after the last PCR cycle. Make sure the dNTPs in the PCR reaction are not exhausted after PCR cycles.
Too much homology	Increase the incubation time to 30 min for homologies over 30 bp. 60 minutes is recommended for homologies greater than 50 bp.
Too much DNA transformed	High concentration of DNA (over 400 ng) in the reaction will either slow down the reaction or compete with your assembled molecules in transformation. Scale to no more than 200 ng per 100 µL chemically competent cells.

2. Problem: There are many colonies after transformation, but none of the plasmids contain inserts.

Possibility	Solution
Incomplete linearization of vector	Digest vector completely; generate incompatible overhangs; gel-purify your digestion product; transform a no-insert control to verify few background colonies can grow.
Contamination of PCR template carrying the same antibiotic resistance	1-10 ng of plasmid template is usually sufficient for PCR reaction. Digest the plasmid template with Dpn I, or gel-purify the PCR product.
Antibiotics expired or incorrect	Do an empty incubation in 37°C to make sure the antibiotics are not expired.

VII. Limited Use License and Warranty

Limited Use License

Following terms and conditions apply to use of Fast-Fusion™ Cloning Kit (the Product). If the terms and conditions are not acceptable, the Product in its entirety must be returned to GeneCopoeia within 5 calendar days. A limited End-User license is granted to the purchaser of the Product. The Product shall be used by the purchaser for internal research purposes only. The Product is expressly not designed, intended, or warranted for use in humans or for therapeutic or diagnostic use. The Product must not be resold, repackaged or modified for resale, or used to manufacture commercial products without prior written consent from GeneCopoeia. This Product should be used in accordance with the NIH guidelines developed for recombinant DNA and genetic research. Use of any part of the Product constitutes acceptance of the above terms.

Limited Warranty

GeneCopoeia warrants that the Product meets the specifications described in the accompanying Product Datasheet. If it is proven to the satisfaction of GeneCopoeia that the Product fails to meet these specifications, GeneCopoeia will replace the Product. In the event a replacement cannot be provided, GeneCopoeia will provide the purchaser with a refund. This limited warranty shall not extend to anyone other than the original purchaser of the Product. Notice of nonconforming products must be made to GeneCopoeia within 30 days of receipt of the Product. GeneCopoeia's liability is expressly limited to replacement of Product or a refund limited to the actual purchase price. GeneCopoeia's liability does not extend to any damages arising from use or improper use of the Product, or losses associated with the use of additional materials or reagents. This limited warranty is the sole and exclusive warranty. GeneCopoeia does not provide any other warranties of any kind, expressed or implied, including the merchantability or fitness of the Product for a particular purpose.

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